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STUDIES ON THE OXIDIZING POWER OF ROOTS[1]

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The present paper embodies a series of studies upon the oxidizing powers of plant roots grown in aqueous extracts of soils and in solutions of various compounds. The results, it is believed, throw some light on the action of plants upon the soil and indicate how soil conditions affect certain functions of the plant.

The experiments which are presented show that plant roots are able to carry on active extra-cellular oxidation, chiefly by means of the enzymes which they secrete. From the standpoint of root excretions the study is of interest because it has often been supposed that the roots of growing plants excrete organic and inorganic acids which aid in the solution of soil minerals. The idea undoubtedly owes its prevalence largely to the experiments of LIEBIG[2] and of SACHS[3] which demonstrated the corrosion of polished marble plates by growing plants. The more recent investigations upon the subject made by CZAPEK,[4] KOSSOWITCH,[5] and KUNZE[6] have shown, however, that very little acid is excreted by the roots of the higher plants, and that the results of the earlier workers were mainly due to the action of carbon dioxid.

This oxidizing power of plants gives them an important action upon the soil. Whether they have the power to oxidize the inorganic constituents of the soil remains to be determined; but it has been shown that they are able to oxidize organic substances, such as the chromogens employed in these experiments, at a fairly rapid rate. If these substances are oxidized, it is only logical to conclude that the organic substances occurring in the soils are also oxidized by the action of plant roots.

This oxidation of organic compounds is of additional interest in the light of recent investigations, which show that the cause of unproductivity in certain soils is due to the presence of toxic organic compounds.[7] The beneficial effect of oxidation in such soils may be inferred from the results of thorough tillage, involving sub-drainage and cultivation, since these operations promote aeration of the soil with subsequent increase in growth of roots and microorganisms. Under such conditions experience has shown that the organic substances in the soil are most completely converted into substances commonly known as humus. It is certain that the oxidizing activities of the soil and plant play a significant part in this important process.

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[1] Published by permission of the Secretary of Agriculture.

[2] Annalen Chem. Pharm. **105**:139. 1858.

[3] Bot. Zeit. **18**:117. 186c.

[4] Jahrb. Wiss. Bot. **29**:321. 1896.

[5] Ann. Sci. Agron. II. **8**:220. 1903.

[6] Jahrb. Wiss. Bot. **42**:357. 1906.

PREVIOUS WORK

The existence of an oxidation process in soils has long been known. Without apparently understanding the precise cause of this phenomenon, LIEBIG pointed out its importance for productive soils, and, according to the same author, the phenomenon had been earlier investigated by INGENHOUSS and DE SAUSSURE. Among the more modern investigations on the processes of oxidation in soils the works of DEHERAIN and DEMOUSSY,[8] WOLLNY,[9] RUSSELL,[10] and DARBISHIRE and RUSSELL[11] should be mentioned. These investigations are all along the line of bacterial activities in the soils. Recent studies by KÖNIG,[12] however, furnish evidence of a catalytic power of soils due to the presence of an enzyme.

The literature dealing with the oxidizing power of plant juices is already voluminous. Within recent years, our knowledge of processes going on within the plant has been greatly extended by the studies which have been made upon oxidizing enzymes. Since several comprehensive and instructive summaries of work on this subject have appeared, among which may be mentioned those of BACH and of CZAPEK'S *Biochemie*, it is unnecessary in the present paper to review what has been done in this rapidly developing field.

[7] Jour. Amer. Chem. Soc. **30**:1295, 1599. 1908. Bur. Soils, U. S. Dept. Agric. Bulls. 36, 40, 53.

[8] Annales Agron. **22**:305. 1896.

[9] Die Zersetzung der organischen Stoffe und die Humusbildung. Heidelberg. 1897.

[10] Jour. Agr. Sci. **1**:261. 1905.

[11] Jour. Agr. Sci. **2**:305. 1907.

[12] Landw. Versuchsst. **63**:471. 1906; **66**:401. 1907.

The study of the oxidizing enzymes which are given off by the roots of plants, i. e., extra-cellular oxidation, has received less attention, and it is to this particular field that the present study belongs.

Molisch[13] appears to have been the first to demonstrate the oxidizing power of root secretions and to show their enzymotic nature. He found that the root secretion was capable of oxidizing various organic substances, such as guaiacol, pyrogallol, and gallic acid. His work showed that there was considerable active secretion on the surface of growing roots, and that this secretion had definite powers to effect changes in organic substances.

Czapek,[14] in making a general study of root secretions, followed some of the investigations made previously by Molisch. From experiments upon the action of seedling roots upon starch paste and sugar solutions, he regarded it probable that the growing roots produce only diastase or inverting ferments, although exact proof could not be offered. He believed, however, that the experiments of Molisch failed to prove the production of oxidizing enzymes by roots.

The ideas of the oxidizing powers of roots set forth by Molisch are well corroborated by the investigations of Raciborski[15] upon the oxidizing powers of plant tissues.

In his work reagents were used which were so nearly non-toxic that they could be added to solutions in which plants were grown. In some experiments the reagents were added to water cultures containing the growing plants; in others, strips of filter paper which had been saturated with the reagent were applied to the surface of growing roots. The substances used for showing the oxidizing power of growing roots were α-naphthylamine, benzidine, phenolphthalin, ferrous ammonium sulfate, Barbadoes aloes, guaiac, phloridzin, pyrogallol, leucomethylene blue, etc.

The extra-cellular oxidation by the roots of the phanerogams studied was found to be strongly localized and limited to the absorbing surface of the root. The most intensive oxidation occurs in the region covered by the root hairs. After the death of the root hairs,

[13] Sitzb. Akad. Wiss. Wien. Math. Nat. Kl. **96**:84. 1888.

[14] Jahrb. Wiss. Bot. **29**:321. 1896.

[15] Bull. Acad. Sci. Cracovie **1905**:338, 668, 693.

as the root grows older, the oxidation becomes weaker (as shown by the less intense coloration) and vanishes in basipetal order. The short growing zone of the root between the root cap and the region of root hairs shows very little if any oxidation. The cells of the root cap behaved differently in different plants. In some there was a very weak oxidizing power, insignificant in comparison with that of the absorbing region of the root; in other plants the root cap showed no power to oxidize. This observation is the more interesting because PFEFFER[16] regarded the experiments of MOLISCH to lack proof that the guaiac-bluing power was due to living cells and not to the dead or dying cells of the root cap.

The oxidation which occurred in naphthylamine and benzidine solutions first appeared on the outer surfaces of the walls of the root hairs and epidermal cells, later in the wall itself, and finally in the outer layer of the ectoplasm. When roots were left for a long time in a solution of these chromogens, the entire protoplasm of the epidermal cells and root hairs gradually assumed the dark color of the oxidized chromogen, although it was not determined whether this color was due to the diffusion inward of the dye formed at the surface, or to an actual intracellular oxidation.

MATERIAL AND METHODS

The experiments described in succeeding pages consisted in studying the oxidizing power of wheat plants grown under various conditions in connection with soil-fertility investigations. It was necessary to grow the wheat plants used for experimentation in solutions, since in such cultures it is possible to observe the oxidation without disturbing the roots. For the study of soil conditions an aqueous extract was made by stirring one part of soil with five parts of distilled water for three minutes and filtering after 30 minutes through a Pasteur-Chamberland clay tube. It has been found that soil extracts prepared in this manner possess a plant-producing power similar to that of the soil from which they were made. In other words, fertile soils yield extracts which promote good plant growth, and infertile soils give extracts producing poor plant growth.

The water used in making solutions and soil extract was the ordinary laboratory distilled water treated with carbon black. The water was distilled from a copper boiler, condensed in a block-tin worm, and collected in a tin-lined copper tank. This method of distillation gives very good water for ordinary chemical work, but does not free it from traces of volatile organic compounds, which may exert a toxic action as was described by LIVINGSTON[17] and the writers.[18] It has been found that these deleterious substances may be effectually removed by treating the distilled water with some finely divided solid which possesses a strong absorbing power, such as ferric hydrate, or carbon black. The procedure usually followed was to shake up a small quantity of the carbon black in the water and let it stand for 30 to 60 minutes; at the expiration of that time the water was filtered through ordinary filter paper and was ready for use. This treatment has been found to be as efficient in producing physiologically pure water as redistillation with strong oxidizing agents, like acid potassium bichromate or alkaline potassium permanganate.

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[16] Abhandl. kön. säch. Gesells. Wiss. Leipzig, Math. Phys. Cl. **15**:375. 1889.

The varieties of wheat used in the experiments were "Chul" and "Harvest Queen." The seeds were germinated on floating perforated plates, according to the method described by LIVINGSTON[19] and in Bulletin 40 of this Bureau. The seedlings were transferred from the perforated germinating plates to the cultures just as the first true leaf was beginning to emerge from its sheath.

The plants were held in notches cut in the edge of a cork, as described by LIVINGSTON. In this way the seed in which enzymes were acting upon reserve food materials were kept out of the solutions and the enzyme effects observed were ascribable to substances arising from the roots.

Salt-mouth bottles, having a capacity of 250^{cc}, were used as culture jars, and ten wheat plants were grown in each jar. In each test two cultures containing 20 wheat plants were usually employed, and comparison was made with an equal number of plants growing in pure distilled water under the same conditions. All experiments were conducted in a greenhouse. During the season of the year in which conditions were most favorable for growth, each experiment was conducted for 8 to 12 days before studying the oxidizing action of the plants, but during the cloudy winter weather the time was sometimes extended to 14 to 16 days.

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[17] Bur. Soils, U. S. Dept. Agr. Bull. 36. 1907.

[18] Bur. Soils, U. S. Dept. Agr. Bull. 40. 1907.

[19] Plant World **9**:13. 1906.

In addition to determining the oxidizing power of the plants subjected to various treatments, their growth was estimated by recording the green weight and transpiration of each culture.[20]

SUBSTANCES CAPABLE OF SHOWING THE OXIDIZING POWER

Two classes of substances have been found useful in showing the oxidizing powers of plant roots in solution cultures. The first class comprises certain soluble chromogens, which yield, upon oxidation by the plant roots, insoluble colored compounds mainly deposited upon the surface of the roots. The oxidation is usually rapid enough to produce marked results before the surface extension of the roots perceptibly disturbs the zonal distribution of the colors. The second class of chromogens consists of certain substances which give soluble coloring matters as the result of the oxidizing action of the roots. The oxidizing action may be shown by the change from a colorless to a colored compound, or by a change from one color to another and distinctly different color.

Compounds belonging to the first class which have been used in this work are α-naphthylamine, benzidine, vanillin, vanillic acid, and esculin.

Alpha-naphthylamine is only slightly soluble in water, but constitutes a good reagent for use in plant cultures because its colorless solution is non-toxic, or nearly so, to plants. When oxidized by the roots of plants, or by reagents such as ferric chlorid or silver nitrate, α-naphthylamine is converted into the insoluble, lavender-purple oxynaphthylamine. When the oxidation is performed by the growing roots of a plant, the oxynaphthylamine is deposited upon the surface of the roots in characteristic zones, as already described by RACIBORSKI (*op. cit.* 357). The root cap is slightly if at all colored; the zone of primary meristematic cells immediately back of the root cap is marked by a distinct narrow band of color; the zone of actively growing cells in the region of greatest elongation is not intensively colored; the more slowly growing portions of the root possess the purplish color, but it becomes less intense as one passes to the upper parts of the root.

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[20] For a discussion of the value of these criteria the reader is referred to LIVINGSTON, BOT. GAZETTE **40**:178. 1905; JENSEN, BOT. GAZETTE **43**:11. 1907; and Bureau of Soils, U. S. Dept. Agric. Bull. 47. 1907.

The superior oxidizing power of the meristematic tissues of the plant is not only shown by the narrow zone of deep color formed on the primary meristem of the apical portion of the roots, but also by the small dots of color produced on that portion of the root from which secondary roots arise. If a wheat root 8 to 10^{cm} in length is placed in a solution of naphthylamine it will exhibit, in addition to the deeper colored zones near the apex, dark-purple spots at the places where secondary roots are forming and are about to break through the cortical layers of the primary root. If secondary roots are already present they show the same zones of colors already described for the primary roots.

The concentrations of naphthylamine used in solution cultures are necessarily low on account of its slight solubility in water, but are sufficiently strong to show the oxidation. In ordinary practice 10 parts of naphthylamine to a million (10^{mg} per liter of water) is a suitable concentration to use. This concentration will eventually retard the growth of wheat plants, but is not detrimental to growth in the length of time usually required to demonstrate the oxidizing powers of the plant roots. A concentration of 5 parts per million sometimes acts as a stimulant to growth.

Benzidine is another chromogen, which is oxidized by plants and may advantageously be used to demonstrate their oxidizing action. It is only slightly soluble in water, but in weak, colorless solution it is readily oxidized by plant roots to an insoluble dye which gives the roots a blue-black or black appearance. Benzidine is slightly toxic to plant growth, but does not cause pathological conditions within the time required for demonstrating the oxidizing power of the plant roots. A concentration of 5 parts benzidine to a million of water will give good results and does not injure wheat roots in 24 hours, although that concentration may eventually inhibit growth.

The effect of oxidation may easily be demonstrated by allowing the roots of wheat plants to grow in a 5 parts per million solution of benzidine for 12 to 24 hours. The formation of colors in distinct zones is fully as striking as in the case of α-naphthylamine. As before, the root cap does not produce oxidation products, the primary meristem is marked by a narrow band of brown color, the zone of elongation is practically uncolored, whereas the portion of the root just above the zone of greatest elongation is entirely colored blue-black or black by the oxidation products.

Solutions of vanillin and vanillic acid act in much the same manner as those of naphthylamine or benzidine, but the concentrations required to demonstrate the oxidizing power of roots are quite strongly toxic.[21] Both substances are converted by the oxidizing action of the roots into a purple insoluble dye which stains the surface of the roots in the manner previously described. The concentration of vanillin in the solution most favorable for showing oxidation with wheat plants lies between 250 and 500 parts per million. A solution of this concentration will demonstrate the oxidizing power of the roots before the plants become seriously injured. To demonstrate the oxidizing power of roots with vanillic acid, a solution of the latter containing 25 to 50 parts per million should be used.

Esculin is another chromogen belonging to this class, but was found to be less suitable for this work. Esculin solutions, when freshly prepared, exhibit a blue fluorescence. After plant roots have grown for a few days in such a solution, the blue fluorescence is lost, and the roots themselves are colored yellow as a result of their oxidizing activity, the dye formed being insoluble and remaining upon the surface of the roots where the greatest oxidation occurs. The concentrations necessary to demonstrate the oxidizing power of roots range from 500 to 1000 parts per million, and are eventually quite toxic to wheat plants.

The second class of chromogens, viz., those which are converted into soluble coloring matters, are in many respects more useful for oxidation studies than those belonging to the first class, because the intensity of the color, and hence the amount of oxidation, can be quantitatively expressed. The substances belonging to the second class which have been employed in this study are phenolphthalin, aloin, and leuco-rosolic acid. Alcoholic solutions of guaiac were also used for various tests, but could not be put into solution cultures containing growing roots.

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[21] Bur. Soils, U. S. Dept. Agric. Bull. No. 47. 1907. Proc. Soc. Biol. Chem 1:33. 1907. BOT. GAZETTE 45:73. 1908.

The value of phenolphthalin, a leuco-compound prepared from phenolphthalein, as an indicator of oxidizing enzymes, has been demonstrated for plant work by KASTLE[22] and by RACIBORSKI.[23] Phenolphthalin is prepared by the method described by BAEYER,[24] which consists in reducing ordinary phenolphthalein with zinc dust and sodium hydroxid to phenolphthalin. The latter substance is oxidized back to phenolphthalein by the oxidizing power of the plant roots, a change which is readily demonstrated when the solution is rendered alkaline. The following procedure was observed in preparing this reagent. Weigh out 250^{mg} of phenolphthalein, 3^{gm} of sodium hydroxid, and 4 or 5^{gm} of zinc dust. Place all in a flask and add 100 to 150^{cc} of water. Place the flask on a sand bath and heat sufficiently to cause a rapid evolution of hydrogen, without causing the contents of the flask to boil violently. The heating usually requires 2 to 3 hours to effect reduction of the phenolphthalein. The contents of the flask, after reduction is completed, may be filtered and rendered nearly neutral with hydrochloric acid, and may then be used as an indicator in the plant cultures. However, better results may be obtained by using phenolphthalin purified according to the method given by BAEYER. After purification the phenolphthalin is dissolved in $N/10$ or $N/20$ NaOH and a few cubic centimeters of the alkaline solution put into each culture, adding equal amounts to cultures which are to be compared. If quantitative results are desired, it is necessary to reduce all the solution cultures to neutrality or the same degree of alkalinity. A very slight degree of alkalinity is not usually harmful to plants within the duration of an experiment, and is favorable to the process of oxidation. Phenolphthalin is slowly oxidized by mere contact with the air; therefore it is advisable to instal controls which will allow the results to be corrected for this atmospheric oxidation. When the phenolphthalin is added to the solution cultures, a like quantity is therefore added to jars of distilled water equal in volume to the cultures. The amount of oxidation in these blanks is subtracted from what is observed in the plant cultures.

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[22] Amer. Chem. Jour. **26**:526. 1901. Hyg. Lab. U. S. Pub. Health and Mar. Hosp. Ser. Bull. 26. 1906.

[23] Bull. Acad. Sci. Cracovie, Math. Nat. Cl. **1905**:338.

[24] Annalen Chem. **202**:80. 1880.

Plant cultures usually show striking results at the end of 10 to 20 hours, depending somewhat upon the temperature and amount of root surface. At the end of the experiment the plants are removed from the cultures, and all are rendered distinctly alkaline with sodium hydroxid solution, and thus the red phenolphthalein color appears.

The great advantage in the use of phenolphthalin to demonstrate the oxidizing power of roots lies in the fact that it is capable of yielding quantitative results. After the colors have been developed in the alkaline solution, their intensities may be estimated by the aid of a colorimeter. In the work reported below the color intensities were estimated by means of the colorimeter previously described,[25] which permits of rapid and accurate readings. The colored solutions may be read against a standard phenolphthalein solution or against a standard Lovibond red glass slide.[26] The readings of the colorimetric tubes are inversely proportional to the color intensity and are easily reduced to their relative values.

Aloin is a substance which may be used to demonstrate the oxidizing power of roots in the same way as phenolphthalin is used. Aloin, or barbaloin, is the active principle of Barbadoes aloes, and is obtained in the market in the form of a yellow powder, fairly soluble in water and serving well as an indicator of the oxidizing power of plants. At the concentrations used in our work it was not found to exert any toxic action upon plants. As a result of a limited investigation of the chemistry of aloin, it seems that its value as an indicator of the oxidizing power of plants depends largely upon the content of iso-barbaloin.

When oxidized by the plant roots, the aloin solution is changed from a pale yellow color to a permanent deep wine-red color, similar to that given by KLUNGE'S reaction for iso-barbaloin. KLUNGE'S reaction[27] consists in dissolving aloin (containing iso-barbaloin) in a 15 per cent. sodium chlorid solution and adding 5^{cc} of concentrated copper sulfate solution. Almost immediately the straw-yellow solution begins to change to a permanent deep wine-red. The change is hastened by warming the solution.

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[25] Jour. Amer. Chem. Soc. **27**:1192. 1905. Bur. Soils, U. S. Dept. Agric. Bull. 31, 1906.

[26] LOVIBOND, Jour. Soc. Chem. Ind. **13**:308. 1894; see also SCHREINER, Pharm. Rev. **19**:61. 1901.

[27] Schweizerische Wochenschr. Pharm. **21**:1. 1883; also Leger, Compt. Rend. Acad. Sci. Paris **131**:55. 1900.

When experimenting with plant juices containing enzymes, there appears to be a difference between the reactions to aqueous and alcoholic solutions of aloin. As the result of experiments described in detail in a subsequent section of this paper, it was found that an aqueous solution of aloin is a better indicator of the presence of oxidase, while an alcoholic solution of aloin is the better indicator of peroxidase.

Aloin, like phenolphthalin, should be added to neutral or faintly alkaline culture solutions, and where quantitative results are desired all solutions should be of the same degree of alkalinity. In all of our work aloin was added at the rate of 100^{mg} of aloin to 250^{cc} of culture solution. If actively growing seedlings are used in a very faintly alkaline solution, a small amount of red color may be developed in an hour or two, but the experiments should be continued for 12 to 20 hours for the final observation. When certain inorganic salts were present in the culture solutions, the aloin red color was slightly modified. The addition of nitrates or previous treatment of the soil extracts with an absorbing agent gave the oxidized aloin a purplish tinge, resembling that of fresh fuchsin solution. The presence of calcium carbonate gave a purer red color, resembling alkanna or cochineal solution.

The fact that aloin is changed by oxidation from a light yellow to a deep red solution makes it somewhat more difficult to obtain colorimetric readings than in the case of phenolphthalin, where there is a change from a colorless to a red solution. Nevertheless, it is practical to use the colorimeter for measuring approximately the intensity of color in aloin solutions, by arranging the solutions in the order of their apparent color intensities, and using each solution first as an unknown and then as a standard for the next higher. For example, let No. 1, the weakest color, be the standard against which No. 2 is read. Then discard No. 1; set No. 2 at a convenient mark, and, using it as the standard, read No. 3. In turn No. 3 is used as the standard for No. 4, and so on. In this way one avoids the necessity of comparing a solution strongly tinged with yellow against a solution which contains little or no yellow tint. In any two solutions to be estimated the tints of yellow should not be greatly different.

Leuco-rosolic acid is another reagent which is useful for demonstrating the oxidizing power of plant juices[28] and plant roots. When a few cubic centimeters of a slightly alkaline, colorless solution are added to a culture containing plants, the leuco-rosolic acid is oxidized back to rosolic acid, the change being shown by the appearance of the red color. This reagent is not so generally useful as phenolphthalin and aloin, since it is more readily oxidized by mere contact with the air, as well as being more difficult to prepare.

PRELIMINARY EXPERIMENTS

The first experiments were conducted for the purpose of ascertaining some general facts concerning the phenomenon of oxidation by the roots of seedlings, as well as to learn the methods best suited for studying oxidation in soil extracts. The experiments of RACIBORSKI dealt with plants growing under what may be termed pure culture conditions, and those of KASTLE were concerned with the oxidizing power of plant extracts.

In the first experiment, wheat seedlings 4 days old were placed in solutions of α-naphthylamine having concentrations of 1, 2, 5, and 10 parts per million, and in a solution of 5 parts per million benzidine. The experiment was set up at 4 P. M. August 6, and observations were made eighteen hours later. At the expiration of that time colors could be distinctly seen on the white surface of the wheat roots. The roots in the solution of 1 part per million naphthylamine were pale lavender; in 2 parts per million they were pronounced lavender, except at the root cap; in the 5 parts per million solution they were violet in the region occupied by the primary meristem, and in the region of the root hairs where growth of elongation occurs, while the root cap and a narrow zone just above the primary meristem were uncolored; in the 10 parts per million solution the roots showed the same colors as in that of 5 parts per million. The roots in the solution of 5 parts per million of benzidine showed their power of oxidation by the formation of brown-violet color, distributed in the same manner as described for the roots which grew in the solutions of naphthylamine.

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[28] KASTLE, J. H., Hyg. Lab., U. S. Pub. Health and Mar. Hosp. Serv. Bull. 26: 17. 1906.

In order to learn whether the oxidizing powers of roots were affected by conditions which favor growth, and also whether the method used in the first experiment would show such differences, the following experiment was made. Three water cultures were made, in each of which an equal number of wheat seedlings of uniform age and size were employed. One culture was made with redistilled water, the second with an aqueous extract of a rich garden soil, the third with a dilute aqueous extract of well-decomposed stable manure. After the plants had grown for one day in these liquids, the oxidizing powers of the plants were observed by transferring them to other bottles containing 2 parts per million of α-naphthylamine in distilled water. At the expiration of 18 hours the intensity of the purple colors showed that the roots which had previously grown in the extract of garden soil had oxidized more naphthylamine than those which had grown in distilled water, and those which had grown in manure extract had oxidized more naphthylamine than those from the garden soil extract. At the end of 24 hours the differences in color intensity in the two cultures were still more marked.

The next experiment was an attempt to employ a method which would permit a more accurate quantitative expression of the oxidizing power of the roots. Two cultures of wheat seedlings were grown for 5 days in an extract of unproductive soil, under the same conditions as two other cultures in an extract of rich garden soil. Each culture contained 60^{cc} of the respective soil extract. The oxidizing power of the roots in this experiment was shown by using phenolphthalin. The phenolphthalin was prepared by the method given in a previous paragraph, and 0.4^{cc} of the freshly prepared solution were added to each culture of plants after they had grown 5 days in their respective solutions. Nineteen hours after adding the indicator all plants were removed from the cultures and the solutions rendered alkaline, thus producing the phenolphthalein color. The solutions were brought to the same volume by the addition of distilled water, and the relative amount of oxidation was measured by determining the color intensities of the different cultures.

The two cultures of poor soil gave readings of 40 and 42 divisions on the graduated tube against slide No. 2 (Lovibond system); the two cultures of rich garden soil gave readings of 14 and 24 divisions against slide No. 4 (Lovibond system). Averaging the readings and comparing the intensity of the colors, the oxidation in the poor lawn soil and in the rich garden soil stands in the ratio of 1 to 4, or more exactly as 19 to 82. This result indicated that a procedure based upon this method will give satisfactory quantitative results.

This method was further tested by another experiment in which different beneficial treatments were applied to an extract of the unproductive soil used in the last experiment. The results of the last experiment showed that the oxidizing powers of plants growing in solutions of different physiological properties vary considerably, but left the question open as to how much of the oxidation result might be due to plants and how much to the solution. In the present experiment, therefore, two of the four bottles in each set of solutions were left unplanted, and their oxidizing powers measured along with those of the solutions which contained plants. The treatment employed consisted in adding fertilizer substances in the form of pure chemicals. Calcium carbonate was added at the rate of 2000, and sodium nitrate at the rate of 50 parts per million. The cultures were put up August 24 and allowed to grow until August 28, when the amount of water transpired by each culture was ascertained and 3^{cc} of a freshly prepared phenolphthalin solution added to each bottle. The color of the phenolphthalein was brought out by adding a few drops of strong alkali to each culture, and the intensities of the different solutions were compared in the colorimeter. Table I presents the figures which give the relative amount of oxidation in the planted and unplanted solutions. When the phenolphthalin solution was added to the culture jars, the same quantity was added to a jar of distilled water, which served as a control upon the oxidation incident to contact with atmospheric oxygen. The color intensity of the control was determined and subtracted from each of the other readings.

The plants used in this experiment were quite young, and the experiment was only continued for four days, a period rather too short for the maximum oxidation effect, as shown by subsequent experiments; nevertheless, the results show that the different treatments

TABLE I

Relative oxidizing power of cultures and unplanted solutions of Takoma lawn soil extract with and without the addition of fertilizer ingredients. Oxidizing power of plants grown in distilled water used as the basis of comparison (p.p.m. = parts per million).

No.	Solutions	Relative oxidation
1	Distilled water (planted) } Average	100
2	Distilled water (planted) }	
3	Extract Takoma lawn soil (planted)	88
4	" " " " "	74
5	" " " " (unplanted)	8
6	" " " " "	19
7	" " " " +2000 p.p.m. $CaCO_3$ (planted)	113
8	" " " " +2000 " " "	63
9	" " " " +2000 " " (unplanted)	19
10	" " " " +2000 " " "	25
11	" " " " + 50 p.p.m. $NaNO_3$ (planted)	98
12	" " " " + 50 " " "	63
13	" " " " + 50 " " (unplanted)	24
14	" " " " + 50 " " "	17

affected the oxidizing powers. The plants grown in extracts of poor soil possessed less oxidizing power than the controls in distilled water, but the oxidizing power was increased by the addition of calcium carbonate. The addition of sodium nitrate did not show any marked increase to the oxidation in those solutions in the time of the experiment, although its effect as shown in later experiments is always beneficial to oxidation.

The point to be emphasized in this experiment, which has not been previously brought out, is that the soil extract unplanted possesses a comparatively feeble power of oxidation, as shown by the use of phenolphthalin, and that the addition of calcium carbonate and sodium nitrate slightly increased this small oxidizing power.

An additional experiment was performed, using three different salts in distilled water. The results of this experiment, which are given in Table II, confirm those of the foregoing experiment in the soil extract. The cultures were made in duplicate and the figures represent the averages of each pair.

The enzymotic nature of the oxidizing processes was next investigated, using alcoholic guaiac. When alcoholic guaiac is added to a solution in which wheat roots have been growing for a time, evidence of the presence of peroxidase was obtained, but not of oxidase; however, when young growing wheat roots are treated with a solution of alcoholic guaiac they instantly give a blue color, which deepens when hydrogen peroxid is added. This indicates that the cells of the plant root contain an oxidase, as CZAPEK has shown.[29]

TABLE II

Relative oxidizing power of cultures and unplanted solutions of three nutrient salts. Oxidizing power of plants grown in distilled water used as basis of comparison (p.p.m. = parts per million).

No.	Solutions	Relative oxidation
1..........	Controls in distilled water (planted)	100
2..........	Solution, 50 p.p.m. of NO_3 as $NaNO_3$ (planted)	282
3..........	" 50 " " " (unplanted)	39
4..........	" 35 " K as KCl (planted)	72
5..........	" 35 " " " (unplanted)	36
6..........	" 50 " PO_4 as Na_2HPO_4 (planted)	88
7..........	" 50 " " " (unplanted)	21

A word may be introduced at this place concerning the possible function of bacteria in producing oxidizing ferments which might accomplish some of the effects noted. It is, of course, possible that such organisms existed in the culture employed, since after filtering the extracts no especial precautions were taken to keep them sterile, and microorganisms which were on the roots of the plants would certainly be introduced into the solutions. That these microorganisms were responsible for any appreciable amount of oxidation in the experiments described in this paper is hardly possible. In the first place, the solutions used were not well adapted for a very thrifty development of microorganisms, as was shown by their freedom from turbidity, odors, or other indications. The definite zones of color produced when indicators like α-naphthylamine and benzidine were used, and their close correspondence to definite zones of tissue in the root show that the oxidation is performed only by agents intimately connected with the roots. The colors due to oxidation were most intense on the regions of the root where growth was most active, whereas we would expect that the bacteria, if zonally distributed, would be more abundant on the dying cells of the root cap or on the dismantled cortical layers of the older parts of the root. It seems, therefore, highly improbable that the oxidizing activities of microorganisms can be responsible to any appreciable extent for the results observed.

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[29] Annals of Botany **19**:75. 1905.

OXIDATION IN SOIL EXTRACTS

Following the preliminary experiments already described, further experiments were made to study in more detail the oxidizing power of plants grown in extracts of soil of different character. These experiments were chiefly designed to study the oxidizing powers of plants in extracts of good and poor soils, of extracts treated with absorbing agents, and in distillates of soil extracts.

The difference in oxidizing power of plants in extracts of fertile and infertile soils is shown by the following experiments. In the first experiment, an extract of Takoma lawn soil was compared with an extract of good Leonardtown loam. The former is a very unproductive soil, and the latter is a much better and usually a very productive soil. The oxidizing powers of the plants were determined by adding phenolphthalin to the cultures, after the plants had grown in them for nine days. The growth and oxidizing powers of the plants are shown in Table III, relative to control cultures made in distilled water, which are represented as 100 in each case.

TABLE III

Comparative growth and oxidizing powers of plants in extracts of Takoma lawn soil and good Leonardtown loam. Growth expressed in terms of relative transpiration.

No.	Solutions	Relative growth	Relative oxidation
1	Controls in distilled water	100	100
2	Extract Takoma lawn soil	33	72
3	Extract Leonardtown loam	50	286

In the comparatively short time of this experiment during cloudy winter weather, December 10 to 17, the plant growth as manifested by the figures for transpiration did not have time enough to show the relative productiveness of the two extracts, since it has usually been found that the Leonardtown loam extract produces in 14–18 days better plants than distilled water. The figures do show, however, a much greater oxidizing power in the plants grown in the extract of the more fertile soil, even under these conditions.

Subsequent experiments were performed, the results of which corroborated the foregoing. In each case where growth was good, there was also good oxidation; where growth indicated a poor soil extract, the oxidation was small, as will be seen from Tables IV and V.

TABLE IV

Comparative growth and oxidizing powers of plants in extracts of poor sandy loam and garden loam. Growth expressed in terms of relative transpiration.

No.	Solutions	Relative growth	Relative oxidation
1	Controls in distilled water	100	100
2	Extract poor sandy loam	77	103
3	Extract garden loam	125	275

TABLE V

Comparative growth and oxidizing powers of plants in extract of good and poor oils. Growth expressed in terms of relative transpiration.

No.	Solutions	Relative growth	Relative oxidation
1	Controls in distilled water	100	100
2	Extract Arlington clay loam	75	107
3	Extract Clarksville silt loam	123	133
4	Extract Stockton peat	272	400

In all these experiments where direct comparisons are made between the extracts of soils which were so poor as to give less plant growth than pure distilled water, and other extracts giving materially greater growth than the same, it appears to be unmistakably true that the cultures made in extracts of good, fertile soils possess much greater oxidizing powers than those made in extracts of soils of relatively less fertility.

The next question considered was concerned with the effect of treating the soil extract with absorbing agents. Treating the extracts of a more or less unproductive soil with carbon black or other good absorbing agent is usually beneficial to growth. This response seems to be quite general for all poor soil extracts, although their response to other treatments may be quite different. Previous work in this laboratory[30] has shown that this ameliorating action is due to the removal of deleterious organic substances. Extracts were treated with carbon black or ferric hydrate. The absorbing agent was shaken with the soil extract and filtered off at the expiration of a half-hour, in the same manner as the distilled water used in the experiments was prepared. The relative effects of this treatment upon growth and the oxidizing power of the plants is shown in Table VI, where the effect in the untreated soil extract is in each case taken as 100.

TABLE VI

Effect of treatment with carbon black and ferric hydrate upon growth and oxidizing power of plants grown in extracts of various soils. Growth expressed in terms of relative transpiration.

No.	Soil extract	Relative growth	Relative oxidation
1..........	Arlington clay loam*	100	100
	" " " carbon black treated	124	265
2..........	Takoma lawn soil*	100	100
	" " " carbon black treated	137	100
3..........	Alloway clay*	100	100
	" " carbon black treated	116	117
4..........	Dunkirk sandy loam*	100	100
	" " " carbon black treated	112	280
5..........	Miami silt loam*	100	100
	" " " ferric hydrate treated	171	198
6..........	Marshall clay loam†	100	100
	" " " carbon black treated	216	130
7..........	Clarksville silt loam†	100	100
	" " " carbon black treated	450	227
8..........	Elkton silt loam†	100	100
	" " " carbon black treated	179	317
9..........	Cecil fine sandy loam†	100	100
	" " " " carbon black treated	112	200
10.....	Hagerstown loam†	100	100
	" " carbon black treated	230	500
11..........	Cecil sandy loam†	100	100
	" " " carbon black treated	193	241
12..........	Dutchess silt loam†	100	100
	" " " carbon black treated	110	373
13..........	Poor sandy loam†	100	100
	" " " ferric hydrate treated	170	534
14..........	Garden loam†	100	100
	" " ferric hydrate treated	136	313

* Phenolphthalin used in estimating oxidation.
† Aloin used in estimating oxidation.

It will be noted that in all but one of the soil extracts the effects of the treatment with an absorbing agent strongly increased the oxidizing powers of the plants subsequently grown in the extracts, and the growth of the plants was also increased.

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[30] Bur. Soils, U. S. Dept. Agric. Bulls. 28 36, 40. Jour. Amer. Chem. Soc. **30**: 1295. 1908.

The increased oxidation, as well as the increased growth, points directly to the conclusion that the soil extracts have been so improved by the treatment given as to induce a more active functioning of processes necessary to secure the best conditions for growth. In the single case of No. 2 the growth was increased as a result of the treatment with carbon black, but the oxidation was not. This result was frequently obtained with the Takoma lawn soil; in some cases the oxidizing power was even slightly decreased as a result of treatment with absorbing agents, although growth was increased. No satisfactory explanation has as yet been obtained for this apparently exceptional action. It may be found upon further investigation that the lack of response was due to the presence of matter inhibiting oxidation, which was not removed by the carbon black. This question seems worthy of more study than we have been able to give it.

Extracts of poor soils sometimes contain volatile bodies of a deleterious nature, which can be driven off by boiling and collected in the distillate. The writers have described[31] the behavior of plants grown in such distillates. Where the deleterious bodies are volatile, the distillate usually exhibits the same toxic properties which the original extracts previously possessed, and the residue is correspondingly improved.

To study the effects of these distillates upon the oxidizing powers of the plants the following experiments were made. One liter of such soil extract was placed in a distilling apparatus and distilled until 200^{cc} of distillate had passed over and been condensed. This fluid was made up to 500^{cc} by adding water and designated first portion. When a second 200^{cc} of distillate had been collected, it was likewise made up to 500^{cc} and designated second portion. Cultures were made in each portion, together with controls in pure distilled water. At the end of a week the plants in the different solutions showed marked differences. The plants in the first portion of the distillate were very small and were dying; those in the second portion were much better, in fact, were equal to the controls growing in distilled water. One hundred milligrams of aloin was added to each of the culture bottles, and on the following day the amount of oxidation was noted by comparing the intensity of red color in each culture. The cultures in the first portion showed much less oxidation than either of the other two. The most oxidation appeared to have gone on in the cultures in the second portion, which was slightly in excess of that in the control cultures in pure distilled water.

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[31] Bur. of Soils, U. S. Dept. Agric. Bull. 40. 1907.

The question was studied further, and in a more quantitative manner, by the following experiment. An extract of Elkton silt loam, having a volume of 750^{cc}, was placed in a flask connected with a condenser and distilled. The distillate, amounting to 500^{cc}, was collected in two portions of 250^{cc} each and used as a culture medium in which plants were grown. The residue in the distilling flask, which was diluted to its original volume, was also used for growing plants. For comparison, cultures were also made in the original soil extract. The wheat plants were allowed to grow in the various solutions for 13 days, and then their oxidizing powers were estimated by means of phenolphthalin. The growth and oxidation are shown in Table VII.

TABLE VII

Growth and oxidation in distillate and residue of extract from Elkton silt loam. Growth expressed in terms of relative transpiration.

No.	Solutions	Relative growth	Relative oxidation
1...........	Original soil extract untreated	100	100
2...........	First portion of distillate	53	20
3...........	Second portion of distillate	70	19
4...........	Residue after distillation, diluted to original volume	132	180

These results show that the distillates of this soil extract were less favorable for growth and oxidation than the original untreated soil extract, while the residue from distillation was materially improved. This seems to indicate that the original soil extract, like others which have been investigated,[32] contained a volatile toxic substance which inhibited oxidation by the roots, and that this substance was driven off by the process of distillation, with resulting benefit to oxidation in the residue. Judging from the growth of the plants, the first portion of the distillate contained a larger proportion of this deleterious substance than the second, although this smaller amount appears to be just as deleterious to the oxidizing powers of the roots as the larger amount present in the first portion. The oxidizing power of the plants in the residue was much greater than in the distillates or in the original soil extract.

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[32] Bur. Soils, U. S. Dept. Agric. Bulls. 28, 36, and 40.

Evidently the oxidizing powers of the roots are affected by certain external conditions, since an improvement in the physiological properties of the soil extract results in increased oxidation, and the presence of deleterious bodies results in decreased oxidation.

From the experimental results thus far presented, it appears that the oxidizing power of the soil extracts themselves can be regarded as partly, but not mainly, responsible for the oxidation observed in the experiments. In one of the preliminary experiments reported in Table I, it was shown that the soil extract after filtration through a Pasteur-Chamberland filter tube exhibited some oxidation, even when no plants were growing. It was likewise shown by the results in Table II that certain nutrient salts dissolved in distilled water were able to accomplish a material amount of oxidation without the presence of growing plants. It seems unlikely, therefore, that any considerable amount of oxidation was performed by microorganisms. If we consider the result in this last experiment, where oxidation was increased in the residue from distillation after continued boiling, it seems that any extensive action, not only of microorganisms, but also of enzymes, must be precluded. In the soil, however, it is quite probable that both of these oxidizing factors would come into play, but it is quite certain that the oxidizing power of the roots would accomplish a considerable portion of the oxidation observed.

THE NATURE AND ACTIVITIES OF THE OXIDIZING ENZYMES

Mention has been made in preceding pages of the enzymotic nature of the oxidizing action of the roots and consideration will now be given to the nature of the enzyme or enzymes which bring about the oxidation. So far as known, the oxidation effects observed were entirely due to the action of enzymes and not to the other activities connected with the growth of the roots themselves.

When a few drops of alcoholic guaiac are added to water, or a suitable solution of salts, in which wheat seedlings have grown for several days, there is sometimes a faint blue color, indicating the presence of oxidase, but more often there is no blue color. When a drop of hydrogen peroxid is added, however, the liquid turns blue, giving a color varying from medium to very intense, depending somewhat upon the age of the seedlings, and the number of roots which have grown in the culture. The guaiac-peroxid reaction indicating a peroxidase is confirmed by the reaction to phenolphthalin and aloin, both of which agree in showing the presence of peroxidase. When the roots of a young wheat plant are immersed in an alcoholic guaiac solution, they immediately turn blue, indicating that they are relatively rich in oxidase, although but little oxidase appears in the water in which they grew. This may be due to the retention of oxidase by the root cells during life, but when the outer cells are killed by the alcoholic guaiac the oxidase escapes and becomes evident through its reaction with guaiac. An aqueous extract of crushed roots, shows strong oxidase reaction as well as peroxidase reaction. In the course of a brief examination of different parts of the young wheat plants, it was found that the partially depleted seeds showed a very strong oxidase reaction when guaiac was used, while the peroxidase reaction was relatively less than in the extract of crushed roots.

When the solution in which wheat roots have been grown for some days is boiled for five or ten minutes, and cooled, the oxidase and peroxidase reactions disappear.

The temperature at which the peroxidase is destroyed was determined by heating a culture liquid which showed an active peroxidase action. The culture liquid was heated to successively higher temperatures and held at each for five minute periods. The temperature at which the enzymes appeared to be destroyed was 60° C. or very close thereto.

The culture liquid was examined for enzymes in a series of cultures of different ages to learn whether the enzyme reaction was equally strong in all. Wheat seeds were germinated on perforated cork plates floating on the surface of water in crystallizing dishes of 500^{cc} capacity. When cultures were on hand aged two, three, four, five, six, and seven days respectively, tests were made with guaiac, alcoholic aloin, and phenolphthalin.

The tests with guaiac showed that the oxidase reaction which was weak in the two- and three-day cultures was quite strong at four days. The tests with alcoholic aloin and phenolphthalin showed that the peroxidase reaction was strongest in the six- and four-day cultures, and considerably weaker in each of the others.

Certain phenomena observed in connection with the use of aloin in aqueous and alcoholic solutions, suggested that they react differently with oxidases and peroxidases. Experiments were accordingly installed to test specifically the action of each solution. Two solutions of aloin were prepared: I, 0.250^{gm} of aloin in 50^{cc} of water; II, 0.250^{gm} of aloin in 50^{cc} of 95 per cent. alcohol. One cubic centimeter of aloin solution I or II was added to 5^{cc} of liquid in test tubes, according to the plan shown in Table VIII. The tubes were prepared and aloin added at 2.45 P. M., on January 10, and the observations recorded in the third column of the table were made at 11 A. M. on January 11. The culture liquid when added to the tubes showed no oxidase but good peroxidase reaction with guaiac.

TABLE VIII

Comparative reaction of aqueous and alcoholic solutions of aloin to a liquid containing peroxidase.

Nos.	Solution	Color observed 11 A. M., January 11
1 and 2..........	Unboiled liquid + 1^{cc} aqueous aloin	pink
3 and 4..........	Boiled " + " "	pink
5 and 6..........	Distilled water + " "	faint pink
7 and 8..........	Unboiled liquid + alcoholic "	deep pink
9 and 10..........	Boiled " + " "	yellow
11 and 12..........	Distilled water + " "	yellow

An inspection of these results shows that when only peroxidase is present, aqueous aloin is not particularly applicable for demonstrating the presence of that enzyme in the absence of growing plants, since there was the same development of pink color in the boiled as in unboiled liquid. Alcoholic aloin, on the contrary, was changed to a deep pink in the unboiled liquid, but remained unchanged alike in the boiled liquid and in the distilled water.

The action of the different aloin solutions was next tested in liquids which also possessed a strong oxidase reaction. The roots of 10 wheat seedlings 12 days old were removed and crushed in a mortar with distilled water. The filtered liquid obtained from this source gave a strong reaction for oxidase when tested with guaiac. As before, 1^{cc} of aloin solution I or II was added to 5^{cc} of the root extract in test tubes, according to the plan in Table IX. The tubes were prepared and aloin added at 4.30 P. M. on January 13, and the observations recorded in the third column of the table were made at 11 A. M. on the following day.

TABLE IX

Comparative reaction of aqueous and alcoholic solutions of aloin to a liquid containing oxidase.

Nos.	Solution	Color observed 11 A. M., January 14
1–2–3	Root extract + 1^{cc} aqueous aloin	red
4 – 5	Distilled water + 1^{cc} aqueous aloin	faint pink
6–7–8	Root extract + 1^{cc} alcoholic aloin	pronounced pink
9 –10	Distilled water + 1^{cc} alcoholic aloin	faint pink

The results of these experiments supplement those of the foregoing in which a peroxidase liquid was used, by demonstrating that the oxidase caused a much greater conversion of aloin to "aloin red" with the aqueous than with the alcoholic solutions of aloin. There was in the root extract a distinct peroxidase reaction to guaiac, in addition to the oxidase reaction, and it is therefore only natural that in tubes 6, 7, and 8 there should be some development of color when alcoholic aloin was added.

It is evident, from the above results, that in the absence of living plant roots aqueous aloin is principally a reagent for oxidase and alcoholic aloin for peroxidase. In the experiments where plants are employed it is however needless to say that only aqueous solutions of aloin can be used.

Aloin and phenolphthalin having shown their usefulness as indicators of enzyme action, several other substances were investigated for comparison. Leuco-rosolic acid was prepared by reducing rosolic acid with zinc dust in alkaline solution. When reduction was practically complete, the solution was filtered and neutralized with hydrochloric acid, then rendered slightly alkaline with sodium hydroxid. One cubic centimeter of this solution was added to three different liquids: I, liquid from culture 6 days old; II, the same liquid after having been boiled 10 minutes; III, distilled water. When examined 24 hours later I was pronounced rose-red, while II and III were merely faint pink; which indicates that leuco-rosolic acid is capable of showing the action of those oxidizing enzymes.

Attempts were made to use ferrous ammonium sulfate and potassium iodid as indicators of the oxidizing powers of plants by putting small amounts into cultures containing living plants. Ferrous ammonium sulfate was, in the space of time of the experiment, oxidized by mere contact with the atmospheric oxygen, and was therefore discarded as an indicator. Potassium iodid was not oxidized to free iodin, as RACIBORSKI has also found.[33]

EFFECT OF DIFFERENT CONDITIONS IN THE SOLUTIONS UPON THE ACTIVITY OF THE ENZYMES

Mention has previously been made of instances where the variation in oxidation appeared to be partly due to the acidity or alkalinity of the solution used as a culture liquid. In such cases the growth of the plant roots was affected whenever the alkalinity or acidity was very pronounced. The effect is the more harmful when young seedlings are put into such solutions, because at the beginning of the experiment, when the plants are very tender, the acidity or alkalinity is greatest and gradually diminishes during the progress of the experiment. In investigating the effect of acid or alkaline conditions in the the culture media, instead of using either alkaline or acid solutions at the start, a method was used whereby the originally neutral solutions became acid or alkaline as a result of the selective absorption of the plant in withdrawing nutrients from the solution.[34] It has been demonstrated by KOHN and CZAPEK[35] that fungi may render their culture media alkaline or acid as a result of their selective absorption, whereby an acid or a basic radical is removed more rapidly than the radical to which it is linked. REED[36] has observed a similar action for the higher plants and pointed out its bearing upon the composition of nutrient solutions.

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[33] Bull. Acad. Sci. Cracovie **1905**:668.

[34] See CAMERON, Rept. U. S. Dept. Agr. **71**:67. 1902; Bur. Soils, U. S. Dept. Agric. Bulls. 30 and 41. 1905.

[35] Beitr. Chem. Phys. Path. **8**:302. 1906.

[36] Annals of Botany **21**: 501. 1907.

Solutions were made up from salts whose radicals are differently absorbed by growing plants, e. g., calcium nitrate and potassium sulfate. Where calcium nitrate is furnished the plant takes up NO_3 more rapidly than Ca, with the result that the solution becomes increasingly alkaline. In the case of potassium sulfate, the plants take up K more rapidly than SO_4, with the result that the solution becomes acid. In the experiments which were made upon this problem, an attempt was made to determine the acidity or alkalinity of the solutions when the experiment was terminated. A measured quantity of solution was boiled in a platinum vessel to drive off CO_2, and then titrated. The results of these determinations are shown with the other results in Table X.

TABLE X

Oxidation and growth of wheat plants in solutions which became acid or alkaline as the result of plant growth. Relative growth measured by transpiration.

No.	Solution	Relative growth	Acidity at termination of the experiment	Alkalinity at termination of the experiment	Relative oxidation
1.......	Control in distilled water	100	.....	.....	100
2.......	30 p.p.m. Ca as $Ca(NO_3)_2$.	174	.....	*n*/5000	401
3.......	30 p.p.m. Ca as $CaCl_2$	112	.....	*n*/7500	107
4......	30 p.p.m. Ca as $CaCO_3$	123	.....	*n*/7500	175
5.......	66 p.p.m. SO_4 as $(NH_4)_2SO_4$	46	*n*/7500	.....	102
6.......	66 p.p.m. SO_4 as K_2SO_4	78	*n*/10000	.....	100
7.	100 p.p.m. NO_3 as $NaNO_3$	201	.....	*n*/5000	401
8.......	35 p.p.m. K as KCl	92	Neutral	Neutral	97
9.......	100 p.p.m. NO_3 as $NaNO_3$ *n*/620 63 p.p.m. K as KCl *n*/620	283	.....	*n*/10000	638
10.......	100 p.p.m. NO_3 } as KNO_3 *n*/620 63 p.p.m. K } 63 p.p.m. K as K_2HPO_4 *n*/620	295	.....	*n*/20000	250

These results show that six of the nine solutions became alkaline, two became acid, and one remained neutral. Growth and oxidation were less in the acid solutions than in those which became alkaline, although in the case of calcium chlorid the result was quite low. In the case of potassium sulfate and potassium chlorid a part of the depression may be due to the effect of the potassium, which usually fails to increase oxidation materially, but such is not the case with ammonium sulfate. Neither is it probable that the sulfate radical is the depressing factor, since calcium sulfate compares favorably with calcium nitrate in its effect upon oxidation. The more favorable effect of no. 2 in Table X upon oxidation over nos. 3 and 4 is probably to be attributed to the presence of nitrate, which likewise appears to be responsible for a material increase in growth.

The greater oxidation accomplished by no. 9 over no. 10 is probably not to be attributed to the presence of Cl, but to the smaller amount of K present in no. 9.

On the whole it appears that while oxidation is affected to a certain extent by conditions of acidity or alkalinity arising in the culture medium, it is more materially affected by the specific action of the salts and their elements in the solution.

The direct effect of acid and alkaline conditions upon the activity of peroxidase was investigated by the following experiment in which alcoholic aloin was used as the indicator. A liquid showing strong peroxidase action was taken from a pan in which several hundred 7-day-old wheat seedlings were growing. Various amounts of $n/50$ HCl and $n/50$ NaOH were added to a set of tubes each containing 10^{cc} of the culture liquid and 1^{cc} of alcoholic aloin solution added at 3 P. M., January 13. The following table shows the amount of acid or alkali added in each tube, and gives the record of the colors observed at 11 A. M. the following day.

TABLE XI

Effect of acid and alkaline conditions upon the activity of peroxidase in the absence of plants.

No.	Solution	Color observed at end of 20 hours
1	10^{cc} culture liquid + 0.1^{cc} $n/50$ HCl	faint pink
2	“ “ “ +0.2 “ “	“ “
3	“ “ “ +0.5 “ “	no change
4	“ “ “ +0.7 “ “	“ “
5	“ “ “ +1.0 “ “	“ “
6	“ “ “ +0.1 $n/50$ NaOH	red
7	“ “ “ +0.2 “ “	wine-red
8	“ “ “ +0.5 “ “	deep wine-red
9	“ “ “ +0.7 “ “	“ “ “
10	“ “ “ +1.0 “ “	“ “ “
11	“ “ “ neutral to litmus solution	deep pink
12	“ “ “ “ “ “ “	“ “

From these results it can only be concluded that a slightly alkaline medium is most favorable for this peroxidase reaction. It will be remembered that WOLLNY[37] found also that the oxidation processes in the soil were distinctly favored by slightly alkaline conditions.

The effect of putrefactive processes upon oxidation is another question which was briefly investigated. When a number of seedlings were placed without any support in water containing aloin (the entire root system, seed, and lower part of the plant, being thus submerged), it has been observed that the red color first produced subsequently disappeared. An experiment was accordingly planned to learn whether oxidation phenomena would be affected when the seeds were submerged and gave rise to products of putrefaction. Twelve cultures of wheat plants were prepared and allowed to grow four days in tap water. In one-third of the cultures the seedlings were adjusted in the notched corks so that only the root systems of the plants were submerged; in one-third of the cultures the seedlings were lowered so that the seeds also were submerged, and one-third had the seedlings entirely submerged. On the fourth day 100^{mg} of aloin were added to each culture jar, and they were examined twenty-four hours later with reference to the production of colors. It was found that the cultures planted with only the root systems submerged showed a very considerable amount of oxidation, but in those where the seeds or entire plants were submerged there was none of the red color produced by oxidation. In these cultures where no oxidation was shown, there were putrefactive processes at work, a fact which is taken to mean that the oxidation effects are not observed when putrefactive processes occur. Whether this inhibition of oxidation is caused by the products of putrefaction or by a perverted metabolism, since the plant must function under somewhat anaerobic conditions, remains undecided.

That the oxidizing power of the plant was not destroyed is shown by the fact that by raising the seeds out of the culture water and refilling the jars with fresh tap water containing aloin, the characteristic oxidation occurred.

The foregoing experiments raised a question as to the amount of oxidation which occurs in poorly drained soils, where putrefactive processes are known to exist. In investigating this question, two crops of wheat seedlings were grown in Arlington clay loam in paraffined wire pots, giving the pots different amounts of water.

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[37] Die Zersetzung der organischen Stoffe und die Humusbildung. Heidelberg. 1898.

Lot I of the pots was kept at the optimum water content of the soil. The soil in lot II was kept saturated with water from the start, and the soil in lot III was saturated after the wheat seedlings were up. The relative green weight of the first crop of wheat plants, which grew from February 18 to March 14, was: I, 100; II, 111; III, 104. The relative weight of the second crop, grown from March 17 to April 8, was: I, 100; II, 67; III, 116. Extracts of these soils were then made and wheat plants were grown eleven days in the various extracts. At the end of that time the growth and oxidizing power of the plants in the different solutions were determined with the result shown in Table XII.

TABLE XII

Growth and oxidation in extracts of soils of varying moisture content. Growth expressed in terms of relative transpiration.

No.	Soil treatments	Relative growth	Relative oxidation
1	Soil kept at optimum	100	100
2	Soil kept at saturation	78	69
3	Soil saturated after plants were up	151	11

These results show that the effects of the poor drainage conditions appear to be much more marked upon oxidation than upon growth. The soil, which was kept at optimum and only saturated after the plants had started, seemed to remain favorable to growth in the pots and in the extracts, but its extract was plainly not favorable to oxidation. In regard to the increase of growth, it should be remembered that this lot of soil was alternately very wet and dry during the course of the experiment.

EFFECT OF TOXIC COMPOUNDS UPON OXIDATION

Aside from the foregoing experiments, in which there were used extracts of soils which displayed toxic qualities toward plants, a few investigations were made upon the action of organic compounds whose toxic properties had been previously determined.

The organic compounds employed for this purpose were vanillin, cumarin, and santonin. The compounds were dissolved in distilled water and the resulting solutions used as cultures, taking care that the concentrations chosen were not so great as to be fatal to wheat plants within the duration of the experiment. Vanillin was used at the rate of 100 parts per million, cumarin 10 parts per million, and santonin in a saturated solution, which was nearly 100 parts per million. The growth of the plants, as measured by transpiration and stated in figures, taking the growth of similar plants in distilled water as 100 in each case, was: vanillin, 63; cumarin, 81; santonin, 75. After the plants had grown in their respective solutions for 12 to 14 days, 100^{mg} of aloin were added to each and the results noted on the following day. The results agreed in showing no color indications of oxidation in any of the cultures where the toxic compounds were present, although the roots growing in the control cultures in distilled water showed by the red color produced that a material amount of oxidation had been accomplished.

That the mere presence of organic materials did not inhibit the oxidation was shown by an experiment employing a solution of leucine which was slightly beneficial to the growth of wheat seedlings in solution cultures. Solutions of leucine containing 50 to 100 parts per million, producing an increase in growth over distilled water of 54 and 98 per cent. respectively, were very favorable to oxidation and produced a much deeper aloin red than the cultures in distilled water.

It can only be concluded, therefore, that the toxic organic compounds studied were deleterious to oxidation because of their toxic properties, and it appears that they were even more deleterious to oxidation than to plant growth.

The oxidizing action of the plants upon toxic organic substances is a phenomenon which has been pointed out by the authors in a previous paper[38] and will be referred to again later. The experiments presented in that paper also showed that the addition of sodium nitrate and calcium carbonate to solutions of toxic organic compounds went far toward decreasing their harmful effects, and in some cases overcame them entirely. That the organic salts and the physiological activities of the plants working together had accomplished the destruction of toxic substances, was shown by both plant growth and

[38] Jour. Amer. Chem. Soc. **30**:85. 1908.

chemical tests. It now appears that while this destructive action of the plant upon the toxic body is going on, the oxidizing power in the presence of an excess, as it were, of toxic body is greatly reduced, and may even be entirely inhibited. The conclusion drawn from those experiments was that the plant roots are able to oxidize a certain amount of deleterious organic material, and that the presence of salts which favor oxidation increases the ameliorating action of the plant.

This question was studied a little further by an experiment in which the oxidation in solutions of toxic material was observed. A solution of cumarin containing 10 parts per million, with and without the addition of fertilizer ingredients, was used as a medium for plant growth and subsequently examined for powers of oxidation. Sodium nitrate was added to one portion of the cultures at the rate of 50 parts of NO_3 per million, and calcium carbonate at the rate of 2000 parts per million was added to another portion of the cultures. Wheat plants were installed in the cultures October 7 and grew until October 17. The oxidation was estimated by means of aloin. Table XIII gives the effect of this treatment upon growth and upon the oxidation in the toxic solutions and in control solutions to which no cumarin had been added. In each case growth and oxidation of the plants in distilled water are taken as 100.

TABLE XIII

Effect of sodium nitrate and calcium carbonate on growth and oxidation in solutions of cumarin. Growth expressed in terms of relative transpiration.

No.	Solutions	Relative growth	Relative oxidation
1	Distilled water	100	100
2	" " $+NaNO_3$	196	250
3	" " $+CaCO_3$	170	166
4	Cumarin 10 p.p.m.	81	31
5	" " $+NaNO_3$	159	139
6	" " $+CaCO_3$	110	131

The results of this experiment show, in harmony with the previous ones, that the addition of these fertilizer ingredients overcame to a large extent the deleterious effect of the cumarin upon growth, each one making the cumarin solution to which it was added a better medium for growth than distilled water. An inspection of the figures expressing the relative oxidation shows, however, that the addition of these salts produced relatively greater increases in oxidation than in growth. When sodium nitrate was added to cumarin the resulting growth was twice as great as where only cumarin was present; the oxidizing power, however, was increased over fourfold. In comparison with this effect, it will be noted that the addition of sodium nitrate to distilled water likewise increased the growth twofold and increased the oxidizing powers two and a half times. It seems quite evident, therefore, that the ameliorating powers observed under the conditions of the experiment are to be referred to the increased oxidizing powers which are thereby brought about, and the consequent diminution in amount and activity of the toxic material.

It may be noted that LE RENARD[39] found that nitrates had a greater antitoxic value than other radicals when Penicillium was grown in the presence of copper.

SUMMARY

1. Roots of growing plants exhibit an extracellular oxidizing power which may be demonstrated by the use of suitable chromogens in nutrient solutions or soil extracts.

2. The oxidizing power appears to be most energetic in the region of the root where root hairs are found, and to decrease gradually in activity as that portion of the root becomes older.

3. The oxidizing power of plants grown in extracts of productive soils is greater than that of plants grown in extracts of unproductive soils.

4. Treating the soil extracts with an absorbing agent is usually beneficial to oxidation.

5. The distillate of a poor soil extract which contains volatile toxic compounds was less favorable to oxidation than the residue remaining from distillation.

6. The presence of toxic organic substances in solution was extremely deleterious to the oxidizing power of the plants. The oxidizing power of the plants, especially in the presence of nitrates, was able to alleviate the toxicity of such solutions.

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[39] Essai sur la valeur antitoxique de l'aliment complet et incomplet. Paris. 1907.

7. The process of oxidation is usually accelerated by the addition of sodium nitrate to an aqueous soil extract. The addition of other fertilizer salts also influences oxidation.

8. The process of oxidation by roots is largely, if not entirely, due to the activity of a peroxidase produced by the roots. This oxidizing enzyme is most active in neutral or slightly alkaline solutions. The activity of the enzyme may be inhibited by the presence of acid and also by the conditions in solutions where anaerobic processes occur.

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